stomach: gastric carcinoma (KATO-III); pancreas: ductal carcinoma (SU.86.86) and adenocarcinoma (AsPC-1, Capan-1); ovary: carcinoma (OVCAR-3, OVCAR-5, IGROV-1, SK-OV-3) and adenocarcinoma (OVCAR-8); cervix: carcinoma (HeLa S3, C-33 A, HT-3) and normal primary epithelial cells; uterus: normal primary endometrial cells; kidney: carcinoma (A498, CAKI-1, 786-O, ACHN, RxF393, TK-10, SN-12-C, UO-31) and adenovirus 5–transformed embryonic kidney cells (293); bladder: carcinoma (5637), transitional cell carcinoma (T24), squamous carcinoma (SCaBER), and normal fetal (FHs 738B1); colon: adenocarcinoma (COLO 205, SW-620, HCT-116, HT-29, HCT-15, HCC-2998, KM12R); prostate: adenocarcinoma (PC-3, DU 145), SV40-transformed BPH fibroblasts (BPH-1), normal stromal fibroblasts (31YO), and BPH fibroblasts (S52); central nervous system (CNS): carcinoma (U251, SNB-75) and glioblastoma (SF268); retina: SV40-transformed pigmented epithelium (RPE28SV4); blood: leukemia (Molt4, HEL, SR-WJU, CCRF-CEM, RPMI-8226), T cell leukemia (Jurkats), acute promyelocytic leukemia (HL-60), chronic myelogenous leukemia (K-562), and histiocytic lymphoma (U-937).

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Assembly of Transcriptionally Active RNA Polymerase I Initiation Factor SL1 from Recombinant Subunits

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Initiation of ribosomal RNA synthesis by RNA polymerase I requires the promoter selectivity factor SL1, which consists of the TATA-binding protein, TBP, and three associated factors, TAF_Is 110, 63, and 48. Here the in vivo and in vitro assembly of functional SL1 complexes from recombinant TAF_Is and TBP are reported. Complexes containing TBP and all three TAF_Is were as active in supporting transcription from the human ribosomal RNA gene promoter as endogenous SL1, whereas partial complexes without TBP did not efficiently direct transcription in vitro. These results suggest that TAF_Is 110, 63, and 48, together with TBP, are necessary and sufficient to reconstitute a transcriptionally active SL1 complex.

Transcription of the genes for 28S and 18S ribosomal RNA by RNA polymerase I (RNA pol I) in mammalian cells is a highly regulated process that has been intensively studied for about two decades (*1*). Three components, RNA pol I, the upstream binding factor UBF, and the promoter selectivity factor SL1 are minimally required for accurate initiation of transcription from the human ribosomal promoter (*2*). UBF has been purified to homogeneity and cloned, and recombinant versions of UBF are available (*3*). Until recently the subunit composition and biochemical activities of SL1 were unknown. Recent advances in antibody affinity chromatography allowed us to purify small quantities of SL1 from HeLa (human) nuclear extracts to apparent homogeneity. These studies revealed that SL1 is composed of the TATA binding protein, TBP, and at least three associated subunits of 110, 63, and 48 kD, called TAF_Is (TBP associated factors for RNA pol I) (*4*).

Although reconstitution of SL1 with recombinant TBP and partially purified TAF_I complexes that contain the three subunits obtained by TBP antibody affinity chromatography suggested that these associated factors are important for the assembly of transcriptionally active SL1 complexes (*4*), it was not possible to determine whether these subunits were sufficient to direct promoter and RNA polymerase selectivity. Further purification and disruption of the complex and the reassembly of active SL1 proved to be impractical given the low amount of SL1 in the cell and the limited amounts of purified material obtainable. Thus, it became evident that future advances in determining the structure and function of this essential human transcription complex would require molecular cloning and high level expression of each subunit. In the accompanying paper, we describe the isolation of complementary DNAs (cDNAs) encoding each of the three human TAF_Is present in SL1 (*5*). Here, we describe the assembly of partial and complete SL1 complexes. We have assembled transcriptionally active SL1 in HeLa cells infected with recombinant vaccinia viruses expressing TAF_Is and TBP. As an alternative procedure for reconstituting SL1, we have also reconstructed stable complexes containing the three TAF_Is and TBP in vitro using purified recombinant proteins. After isolating these in vivo and in vitro assembled complexes by antibody affinity chromatography, their ability to support accurate initiation of transcription from the human ribosomal promoter in a reconstituted reaction containing UBF and RNA pol I was tested. Our results suggest that a complex containing TAF_I 110, 63, 48, and TBP is both necessary and sufficient to provide SL1 function.

Using a combination of epitope-tagged versions of the recombinant TAF_Is, we have shown that each of the subunits of SL1 can individually bind to TBP. In addition, the TAF_Is appear to contact each other in order to form a relatively stable complex that presumably involves multiple interactions. These TBP-TAF_I and TAF_I-TAF_I interactions do not require DNA (*5*). However, it was of critical importance to determine whether these protein-protein contacts resulted in a functional SL1 complex that could support transcription of the ribosomal promoter by RNA pol I. Our initial attempts to assemble functional complexes from TAF_Is expressed in *Escherichia coli* or in insect cells infected with recombinant baculoviruses, failed to produce transcriptionally active SL1. One reason for our inability to reconstitute active SL1 complexes was the difficulty in obtaining sufficiently high concentrations of the four subunits of SL1 in order to perform the assembly reactions. The bacterially expressed proteins were largely insoluble, and the amounts of soluble baculovirus expressed TAF_Is were limited. Consequently, we tried alternative means of assembling SL1 from the recombinant subunits. We approached the problem with two complementary strategies: in vivo assembly by co-expression of the subunits and in vitro assembly of denatured and renatured purified subunits.

In order to attempt in vivo assembly of the four components of SL1, we generated recombinant vaccinia viruses that would direct the production of each subunit in HeLa cells upon co-infection with the various combinations of viruses. We constructed the appropriate vaccinia virus vectors such that the recombinant TAF_Is could be distinguished from endogenous TAF_Is on the basis of differences in molecular weight and antibody specificity by fusing a unique epitope tag to each subunit. TAF_I48 was constructed with a FLAG-tag, TAF_I63 was fused to the Polyoma-Myc (PM) epitope, and TAF_I110 was linked to the hemagglutinin-peptide

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(HA) (6). Throughout our experiments, we have used full-length human TBP without any epitope tags or other alterations.

Having generated the recombinant vaccinia virus stocks, each carrying the gene for one of the subunits of SL1, we carried out three separate co-infections of HeLa cells (7). After a period of 18 hours, the cells were harvested and cytoplasmic S100 extracts were prepared. The expression levels of the recombinant proteins were verified by protein immunoblot (Western) analysis (Fig. 1). Approximately equal amounts of each of the overexpressed TAF_Is and TBP were present in the three different extracts that were prepared from the co-infected cells. Except for TBP, endogenous subunits of SL1, which have a mobility distinct from the epitope tagged TAF_Is, were not detected. Recombinant SL1 complexes were antibody affinity-purified from these S100 extracts with the use of one of the epitope tags. Although it was, in principle, possible to use any of the three tags that were engineered into the fusion protein for purification, FLAG-tagged TAF_I48 was most efficiently immunopurified by the use of the M2 antibody covalently linked to beads. Thus, for the experiments described here, we used FLAG 48 as the bait for isolating both complete and partial SL1 complexes. After eluting these different complexes from the M2 beads by the use of an excess of FLAG peptide, we tested their ability to restore promoter selective transcriptional initiation in a reconstituted transcription reaction that contained partially purified RNA pol I and UBF (4).

To ascertain that the different complexes contained the expected complement of TAF_Is and TBP, we first determined the subunit composition of the affinity-purified complexes by protein immunoblot analysis using either epitope-specific monoclonal antibodies (8), or TAF-specific polyclonal antibodies (Fig. 2A). As expected, the complex isolated from HeLa cells co-infected with all four viruses expressing the subunits of SL1, contained FLAG-tagged TAF_I48, PM-tagged 63, HA-tagged 110, and TBP (Fig. 2A, lane 1). The other complexes derived from cells infected with a subset of these viruses each lack either TBP (Fig. 2A, lane 2) or a combination of 63 and 110 (Fig. 2A, lane 3). The protein immunoblot analysis also revealed that in each case only one version of each TAF_I, the recombinant tagged version, could be detected in the isolated complexes which suggests that very little, if any, of the endogenous TAF_I subunits were contaminating these affinity-purified complexes derived from the cytoplasmic fraction of infected cells. Although protein immunoblotting is only semi-quantitative, the amounts of each of the subunits in the different complexes is comparable and, except for HA TAF_I110, very close to that

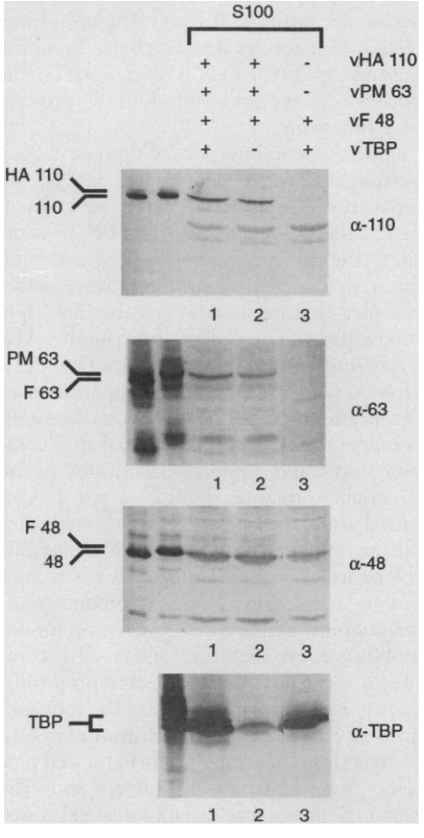

Fig. 1. Overexpression of epitope-tagged subunits of human SL1 in HeLa cells with the recombinant vaccinia virus T7 RNA polymerase co-infection system. HeLa S3 cells were co-infected with vTF7-3 and different combinations of the recombinant vaccinia viruses that carry the genes for hemagglutinin-tagged $hTAF_I110$ (vHa 110), polyoma-Myc–tagged $hTAF_I63$ (vPM 63), FLAG-tagged $hTAF_I48$ (vF 48), and TBP (vTBP) (indicated at the top; lanes 1 to 3) (6). The genes are efficiently transcribed by T7 RNA polymerase and translated in the cytoplasm of the infected cells. Cytoplasmic S100 extracts were prepared from the infected cells (7), and proteins in the extract were separated on 8% (for TAF_I110 and 63) and 10% (for TAF_I48 and TBP) SDS-polyacrylamide gels. Proteins were blotted to nitrocellulose and probed with polyclonal antisera directed against $hTAF_I110$ (α-110), $hTAF_I63$ (α-63), $hTAF_I48$ (α-48), and TBP (α-TBP). For comparison we loaded untagged and tagged versions of the TAF_Is and TBP, expressed in BSC40 cells infected with recombinant vaccinia viruses, on the left-hand side of the gels, as indicated.

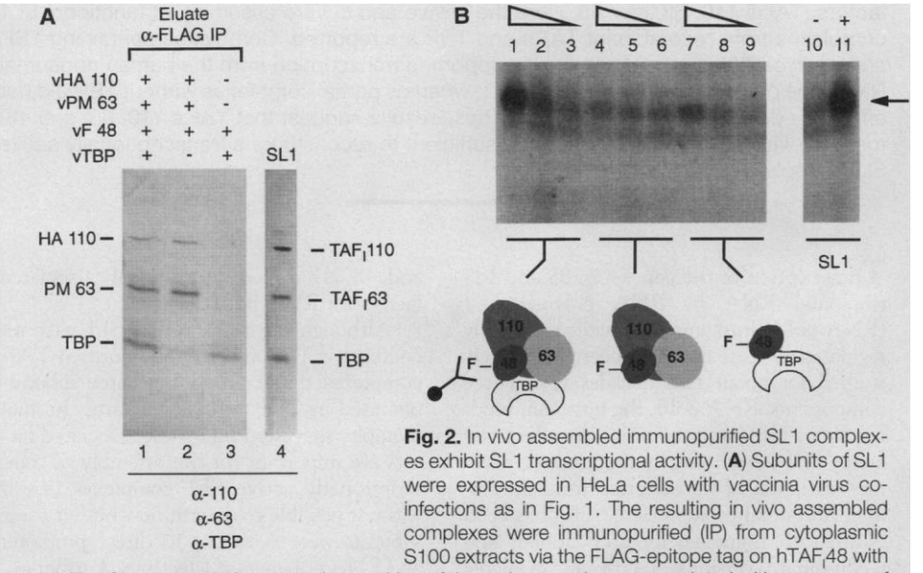

Fig. 2. In vivo assembled immunopurified SL1 complexes exhibit SL1 transcriptional activity. (**A**) Subunits of SL1 were expressed in HeLa cells with vaccinia virus co-infections as in Fig. 1. The resulting in vivo assembled complexes were immunopurified (IP) from cytoplasmic S100 extracts via the FLAG-epitope tag on $hTAF_I48$ with anti-M2(FLAG) resin, and after extensive washing of the resin, complexes were eluted with an excess of FLAG peptide (7). The subunits that co-immunoprecipitated with FLAG 48 and were present in the eluate were separated on an 8% SDS-polyacrylamide gel, followed by blotting and sequential detection with polyclonal antisera directed against the subunits of SL1 (α-110, α-63, and α-TBP). Immunopurified complexes from HeLa cells infected with vHA 110, vPM 63, vF 48, and vTBP (lane 1), from cells infected with vHA 110, vPM 63, and vF 48 (lane 2), and from cells infected with vF 48 and vTBP (lane 3), all in combination with vTF7-3, are shown. Lane 4 contains partially purified SL1 (4). (**B**) The immunopurified complexes shown in (A) were tested in an SL1-dependent in vitro transcription assay (4). Tenfold dilutions of the immunopurified complexes isolated from cells infected with recombinant viruses for the four subunits of SL1 (lanes 1 to 3), from cells infected with recombinant viruses encoding the three TAF_Is (lanes 4 to 6), and from cells infected with only vF 48 and vTBP (lanes 7 to 9), were tested for SL1 transcriptional activity (7). Lanes 10 and 11 show the level of transcription initiation from the ribosomal RNA gene promoter in the in vitro system in the absence (lane 10) and presence (lane 11) of endogenous SL1 purified over heparin agarose and S-Sepharose from HeLa cell nuclear extracts. The amounts of TAF_Is used in the in vitro transcription reactions for lanes 1, 4, and 7, versus lane 11, directly relate to the amounts shown in (A) lanes 1, 2, and 3, versus lane 4.

found in the endogenous SL1 complex (Fig. 2A, compare lanes 1 to 3 with lane 4). Having confirmed the presence or absence of the various recombinant subunits of SL1, we determined the ability of these complexes to provide the essential transcription initiation function attributed to SL1. The complex containing all four subunits (TBP and the three TAF_Is) directed accurate initiation of human ribosomal RNA transcription at a level comparable to a preparation of endogenous SL1 (Fig. 2B, lanes 1 and 11). By contrast, neither the triple complex without TBP (Fig. 2B, lanes 4 to 6), nor the double complex without 110 and 63 (Fig. 2B, lanes 7 to 9) supported transcription. These results suggest that in vivo assembled SL1 with epitope-tagged recombinant TAF_Is can be transcriptionally active. A TAF_I complex without TBP has no apparent transcriptional activity, which confirms a previous finding that TBP is an essential and integral component of SL1 (*4*), even though there is no TATA box, and perhaps no requirement for DNA binding by TBP to the ribosomal promoter.

As a result of the inherent limitations of co-infection experiments in HeLa cells that may contain endogenous pol I TAFs, we turned to in vitro assembly with purified subunits expressed in *E. coli* or from baculovirus. Given the intrinsic insolubility and behavior of the bacterial, as well as baculovirus expressed TAF_Is, we resorted to the purification of the individual TAF_Is from the insoluble aggregates by the use of chaotropic agents, followed by the purification, renaturation, and assembly procedures outlined in Fig. 3A. With the exception of bacterially expressed human TBP, which was purified by conventional chromatography (*9*), the remaining three subunits of SL1 were expressed in *E. coli* (TAF_I110 and 63) or in Sf9 cells infected with recombinant baculoviruses (TAF_I48, which is poorly expressed in *E. coli*) (*10*), and then extracted from the resulting inclusion bodies or precipitates by treatment with 6 M guanidine hydrochloride (Gd HCl).

After the various chromatographic steps in the presence of Gd HCl or urea, each of the TAF_Is was found to be greater than 90% homogeneous as determined by SDS–polyacrylamide gel electrophoresis analysis (Fig. 3B). These three purified TAF_I subunits were combined in approximately 1:1:0.1 stoichiometry (HA 110:His 63:FLAG 48) and dialyzed to remove the urea. One portion of the renatured sample was subsequently mixed with native human TBP (in amounts identical to those for HA 110 and His 63) purified from *E. coli* (Fig. 3B), whereas the other half of the preparation was kept as it was. The resulting complexes, containing or lacking TBP, were isolated away from free subunits with an antibody affinity resin directed against the FLAG-tagged 48 subunit, which is present in limiting amounts. After eluting the quadruple complex containing TBP or the triple complex lacking TBP from the resin with an excess of FLAG-epitope peptide, these protein preparations were tested for promoter-specific transcription in the reconstituted in vitro reaction supplemented with purified RNA pol I and DNA affinity-purified UBF. Addition of the quadruple complex to the in vitro transcription reaction restored high levels of accurate initiation from the human ribosomal promoter (Fig. 4, lanes 6 and 7). The activity of the reconstituted recombinant SL1 complex was comparable to partially purified endogenous human SL1 (Fig. 4, compare lane 1 with 7). By contrast, neither human TBP alone (Fig. 4, lane 3), nor a triple complex containing TAF_I 110, 63, and 48 (Fig. 4, lanes 4 and 5) was sufficient to restore transcriptional activity. These results confirm our previous finding with the in vivo assembled complexes that TBP is an essential com-

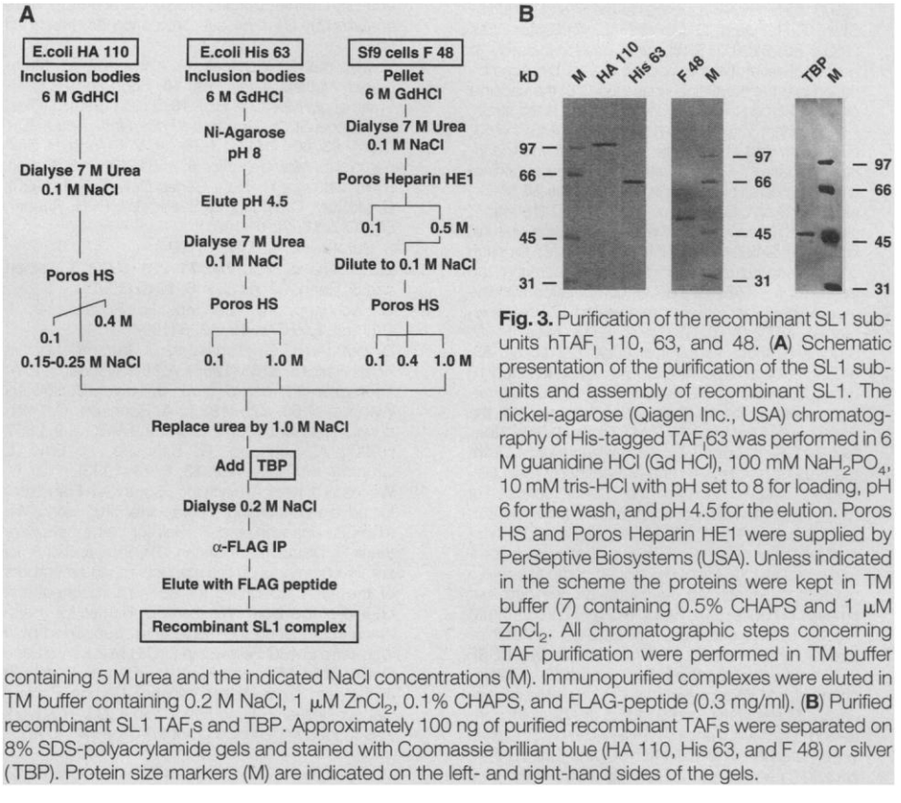

Fig. 3. Purification of the recombinant SL1 subunits $hTAF_I$ 110, 63, and 48. (**A**) Schematic presentation of the purification of the SL1 subunits and assembly of recombinant SL1. The nickel-agarose (Qiagen Inc., USA) chromatography of His-tagged TAF_I63 was performed in 6 M guanidine HCl (Gd HCl), 100 mM NaH_2PO_4, 10 mM tris-HCl with pH set to 8 for loading, pH 6 for the wash, and pH 4.5 for the elution. Poros HS and Poros Heparin HE1 were supplied by PerSeptive Biosystems (USA). Unless indicated in the scheme the proteins were kept in TM buffer (*7*) containing 0.5% CHAPS and 1 μM $ZnCl_2$. All chromatographic steps concerning TAF purification were performed in TM buffer containing 5 M urea and the indicated NaCl concentrations (M). Immunopurified complexes were eluted in TM buffer containing 0.2 M NaCl, 1 μM $ZnCl_2$, 0.1% CHAPS, and FLAG-peptide (0.3 mg/ml). (**B**) Purified recombinant SL1 TAF_Is and TBP. Approximately 100 ng of purified recombinant TAF_Is were separated on 8% SDS-polyacrylamide gels and stained with Coomassie brilliant blue (HA 110, His 63, and F 48) or silver (TBP). Protein size markers (M) are indicated on the left- and right-hand sides of the gels.

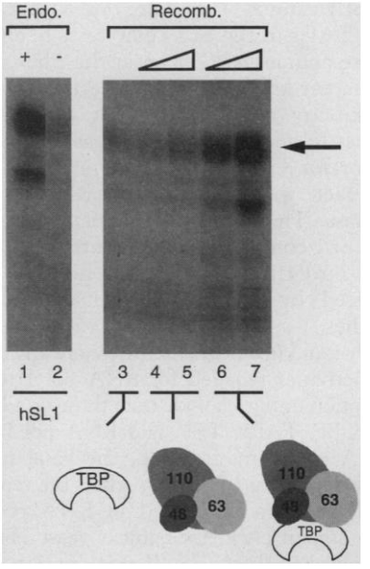

Fig. 4. A complex consisting of the four recombinant subunits of SL1 is necessary and sufficient to reconstitute SL1 activity. Recombinant TAF_I complexes lacking TBP (lanes 4 and 5; fivefold dilutions equivalent to the amounts used in the transcription for lanes 6 and 7), or containing TBP (that is, recombinant SL1; lanes 6 and 7) had been immunopurified by means of FLAG-48 with α-M2 antibody resin and were tested for SL1 activity in an in vitro reconstituted transcription system containing the ribosomal RNA gene promoter template, highly purified RNA pol I, and DNA affinity-purified UBF (*10*). Lane 3 shows a transcription reaction in the presence of TBP only. Transcription reactions in the presence (lane 1) or absence (lane 2) of hSL1 partially purified from HeLa nuclear extracts (that is endogenous SL1) (*4*). Lanes 5 and 7 show the transcriptional activity with reactions containing 20 to 40 ng of each of the TAF_Is, whereas the amounts of TAF_Is in endogenous SL1 (lane 1) is only a few nanograms.

ponent of SL1. These results also suggest that a complex containing TBP and the three TAF_Is is sufficient to reconstitute SL1 activity and accurate RNA pol I transcription initiation because all of these subunits were extensively purified either from *E. coli* or Sf9 cells and are thus unlikely to be contaminated with endogenous TAF_Is.

We previously reported the molecular cloning and biochemical characterization of the enhancer binding factor UBF (*3*), and now we describe the biochemical composition and transcriptional properties of the selectivity factor SL1. UBF and SL1 bind cooperatively to upstream promoter sequences and form a complex that directs transcription initiation (*2*). It is likely that UBF bound to DNA communicates with SL1 by means of direct contact with one or more subunits of the SL1 complex. The UBF-SL1 DNA binding complex also displays selectivity for promoter sequences in a species-specific manner (*2*, *11*). However, UBF can function across species whereas SL1 cannot. Therefore, one or more of the TAF_Is in the SL1 complex is likely to make contact with DNA at the ribosomal promoter and help determine the species specificity of promoter utilization by RNA pol I. In addition to serving as a co-activator for UBF, SL1 is also expected to interact with subunits of RNA polymerase. The role of individual TAF_Is in the SL1 complex in the recruitment RNA pol I to the promoter can now be addressed by direct biochemical binding studies.

It is unknown whether there are any other activities required for RNA pol I transcription initiation aside from those provided by UBF, TAF_Is, TBP, and RNA pol I. In RNA pol II transcription, the basal transcription factor IIB among others is essential and has been implicated in RNA pol II recruitment (*12*). A subunit of yeast TFIIIB involved in RNA pol III transcription is a TBP-associated factor called BRF, which shows similarity to TFIIB (*13*). Thus, TFIIB may serve a common function required by all three classes of eukaryotic RNA polymerases. Although such a function could be served by one of the TAFs in the SL1 complex, none of the SL1 subunits show sequence similarity to TFIIB or BRF. It will be important to determine whether a TFIIB-like activity is also required for RNA pol I transcription. In extracts derived from rodent cells, there are a number of activities that co-fractionate with RNA pol I that have been reported to be required for transcription. One such activity, TIF-IC, has been suggested to serve an analogous function to TFIIF, which directs transcription by RNA pol II (*14*). Another factor, termed TIF-IA, has been implicated in growth regulation of ribosomal RNA synthesis (*15*). With recombinant UBF and SL1 available, it should be possible to screen for other potential ancillary factors required for transcription initiation mediated by RNA pol I in the RNA pol I fractions. Recombinant UBF, TAF_Is, and TBP are valuable tools for dissecting the mechanisms that regulate transcription by RNA pol I of the ribosomal genes in eukaryotic cells.

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6. The $hTAF_I$ 110, 63, and 48 cDNAs (*5*) were modified by polymerase chain reaction to construct Nde I or Nco I sites at the NH_2- termini. The methionine start codon of $hTAF_I$110 and 48, and the very NH_2-terminal residue of $hTAF_I$63 were fused in frame to either the FLAG, HA (hemagglutinin), or to PM (Polyoma-Myc) epitopes. Subsequently, the tagged and untagged genes were ligated as Nco I–Xho I ($hTAF_I$110), or as Nco I–Bam HI ($hTAF_I$63 and 48) fragments to pTM1 [B. Moss, O. Elroy-Stein, T. Mizukami, W. A. Alexander, T. R. Fuerst, *Nature* **348**, 91 (1990)] digested with Nco I–Xho I or Nco I–Bam HI, yielding plasmids pTM-HA 110, pTM-110, pTM–PM 63, pTM–FLAG 63, pTM–FLAG 48, and pTM-48. In a similar manner, the Nco I–Bam HI fragment from pTβ-hTBP was ligated into pTM1, to yield pTM-hTBP. These pTM derived constructs with the T7-promoter regulated TAF_Is and TBP genes and the encephalomyocarditis virus internal ribosome entry sequences at the 5′ end [O. Elroy-Stein, T. R. Fuerst, B. Moss, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6126, (1989)], were used separately to construct recombinant vaccinia viruses by recombination into the thymidine kinase locus of the vaccinia virus wild-type New York City Board of Health strain. Recombinant viruses (vHA110, v110, vPM63, vF63, vF48, v48, and vTBP) were selected in thymidine kinase negative 143 cells with 5-bromodeoxyuridine, and amplified to high titers in BSC-40 cells [M. Mackett, G. L. Smith, B. Moss, *J. Virol.* **49**, 857 (1984)].
7. HeLa S3 cells were grown in suspension culture, infected at a multiplicity of infection (moi) of 5 for each of the recombinant vaccinia viruses carrying the genes for the TAF_Is or TBP, in combination with the recombinant vaccinia virus expressing T7 RNA polymerase (vTF7-3) [T. R. Fuerst, E. G. Niles, F. W. Studier, B. Moss, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8122 (1986)] at a moi of 15. Cells were harvested 18 hour post-infection. Cytoplasmic and nuclear extracts were prepared as described, except that the S100 extract contained 0.2 M KCl and 0.01% Nonidet P40. SL1 complexes were immunopurified from the S100 extracts with anti-M2 antibody resin (IBI, Kodak) directed against the FLAG epitope of $hTAF_I$48. One milliliter of S100 extract (equivalent to approximately 1.3×10^8 HeLa cells) and 20 ml of antibody resin were gently mixed continuously for 4 to 6 hours at 4°C. Antibody-antigen complexes were pelleted by low-speed centrifugation, washed four times in TM buffer [50 mM tris-HCl (pH 7.9), 12.5 mM $MgCl_2$, 10% glycerol, 0.5 mM EDTA, 1 mM dithiothreitol, and protease inhibitors: 0.1 mM AEBSF [4-(2-aminoethyl)-benzene sulfonyl fluoride; Calbiochem], 1 mM sodium metabisulfite, 10 mM Leupeptin, 1 mM Pepstatin, and 5 mg/ml of Aprotinin] containing 0.4 M NaCl and 0.1% NP40, and complexes were eluted in TM with 0.1 M NaCl and 0.02% NP40 containing an excess of FLAG-epitope peptide (0.2 mg/ml) and 0.2 mg/ml of insulin as carrier. The eluate was separated from the antibody resin by centrifugation over a 0.22-μm filter (Millex-GV4, Millipore). The eluate was analyzed for recombinant TAF_Is and TBP by protein immunoblotting and tested for SL1 activity in a SL1-dependent in vitro transcription system containing the human ribosomal RNA promoter template, prHu3, and a 0.4 M heparin flow through 0.2 M eluted DEAE fraction from HeLa nuclear extracts containing RNA polymerase I and UBF, which is depleted of SL1 activity (*4*). Transcripts were analyzed by S1 nuclease protection (*4*).
8. J. C. B. M. Zomerdijk and R. Tjian, data not shown.
9. TBP was purified first on phosphocellulose [M. G. Peterson, N. Tanese, B. F. Pugh, R. Tjian, *Science* **248**, 1625 (1990)] and then loaded in 0.3 M NaCl onto a Heparin column, which was developed with a linear gradient from 0.3 to 0.8 M NaCl. The peak fractions were collected (0.6 M NaCl).
10. For *E. coli* expression of SL1 TAF_Is, the epitope tagged $hTAF_I$110 cDNA was cloned as Nco I–Kpn I into the Nco I–Kpn I sites of pET-3a [A. H. Rosenberg *et al.*, *Gene* **56**, 125 (1987)] and $hTAF_I$63 cDNA was cloned as an Nde I–Bam HI fragment in pET-19b (Invitrogen) resulting in a 10× His-tag at the NH_2-terminus of TAF_I63. Protein expression was induced with 1 mM IPTG for 2 hour at 37°C in *E. coli* strain BL21(DE3). FLAG-tagged TAF_I48 was expressed in Sf9 cells infected with recombinant baculovirus as described in the accompanying paper (*5*). Recombinant TAF_Is proteins were purified from *E. coli* inclusion bodies or from the Sf9 cell insoluble TAF_I48 fraction as schematically depicted in Fig. 3A. Prior to immunoprecipitation, proteins were passed through a 0.22-μm filter. The immunoprecipitation was performed in TM buffer containing 0.2 M NaCl, 0.1% of the zwitterionic detergent CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) as in (*7*) and eluted with FLAG-peptide (0.3 mg/ml). A typical assembly reaction contained 600 ng of F 48, and 10 μg of each of the proteins HA 110, His 63, and TBP. In vitro transcription reactions contained, in addition to the recombinant SL1 complexes and ribosomal RNA gene promoter template, highly purified RNA pol I (H. B. and R. T., unpublished results) and DNA affinity-purified UBF (*3*). Transcripts were analyzed by S1 nuclease protection as described (*4*).
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